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(54)【発明の名称】新規微生物

(57)【要約】

【課題】　有機性／生物性汚泥の固形分を可溶化する性能を有するバチルス・ステアロサーモフィラス(Bacillus stearothermophilus) に属する新規微生物を提供する。

【解決手段】　余剰汚泥やデンプン排水などの有機性／生物性汚泥の固形分を特異的に可溶化する性能を備えたバチルス・ステアロサーモフィラス(Bacillus stearothermophilus) に属する新規微生物、バチルス・ステアロサーモフィラス SPT2-1 (Bacillus stearothermophilus SPT2-1)。

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【特許請求の範囲】

【請求項１】　有機性汚泥あるいは生物性汚泥の固形分を可溶化する性能を有する、バチルス・ステアロサーモフィラス(Bacillus stearothermophilus) に属する新規微生物。

【請求項２】　前記有機性汚泥あるいは生物性汚泥が、

A．形態的性質

(1) 細胞の形：	桿菌
(2) 運動性の有無：	＋
(3) 胞子の有無：	＋
(4) グラム染色：	±

B．培地における生育状態

(1) 標準寒天平板培養：	＋
(2) 標準ブイヨン培養：	＋
(3) 標準ゼラチン穿刺培養：	－
(4) リトマス・ミルク：	＋

C．生理学的性質

(1) グラム染色性：	＋
(2) 硝酸塩の還元能：	＋
(3) 脱窒反応〔硝酸塩ブイヨンでのガス産生〕：	＋
(4) ＭＲテスト：	＋
(5) ＶＰテスト：	－
(6) インドールの生成：	－
(7) 硫化水素の生成：	－
(8) デンプンの加水分解：	＋
(9) クエン酸の利用〔Simmns培地,Christensen培地共〕：	－
(10)無機窒素源の利用〔硝酸塩、アンモニウム塩〕：	＋
(11)色素の生成：	－（非水溶性）
(12)ウレアーゼ：	－
(13)オキシダーゼ：	＋
(14)カタラーゼ：	＋

(15)生育の範囲：　温度；　45～70℃／　ｐＨ；　5.0～ 7.0

(16)酸素に対する態度：　　好気性～通性嫌気性

(17)Ｏ－Ｆ試験〔Hugh leifson法〕：　フラクトース（＋）

(18)糖類の利用と酸およびガス生成：

【表１】

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下水余剰汚泥である請求項１に記載の新規微生物。

【請求項３】　前記有機性汚泥が、デンプン廃液である請求項１に記載の新規微生物。

【請求項４】　前記バチルス・ステアロサーモフィラスに属する新規微生物が、以下の菌学的性質、すなわち；

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糖	酸			ガス	
	24時間	48時間	7日	48時間	7日
Lーアラビノース	−	−	−	−	−
Dーキシロース	−	−	−	−	−
Dーグルコース	+			−	−
Dーマンノース	−	+		−	−
Dーフラクトース	−	−	−	−	−
Dーガラクトース	−	−	−	−	−
マルトース	−	+		−	−
シュークロース	−	+		−	−
ラクトース	−	−	−	−	−
トレハロース	−	+		−	−
Dーソルビトール	−	−	−	−	−
Dーマンニトール	−	+		−	−
イノシトール	−	−	−	−	−
グリセリン	−	+		−	−
デンプン	−	−	−	−	−

の菌学的性質を有する請求項１に記載の新規微生物。

【請求項５】　前記バチルス・ステアロサーモフィラスに属する新規微生物が、バチルス・ステアロサーモフィラス(Bacillus stearothermophilus) SPT2-1[FERM P-15395] である請求項４に記載の新規微生物。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、有機性／生物性汚泥の固形分を効率的に可溶化する性能を備えた新規微生物に関する。

【０００２】

【従来の技術および発明が解決しようとする課題】従来より、下水処理場ならびにし尿処理場などの事業所から排出される生物性汚泥、あるいは食品工場などから排出される有機性高濃度汚泥の処理方法として、細菌などを汚泥に作用させて生物学的に汚泥を分解処理する、次のような有機性汚泥の可溶化のための方法ならびに該方法への応用が期待される菌株がこれまでに報告されている。

【０００３】例えば、酵母エキス残渣を特異的に分解する酵素を生産する菌株、オエルスコフィア属に属する細菌(Oerskovia sp.24 (FERM P-13692):特願平５－211081号参照) を用いた酵母エキス残渣の処理方法（特開平７－184640号参照）；下水汚泥コンポストから好気的にかつ高温条件下で単離した、65℃の至適生育温度を有する、バチルス・ステアロサーモフィラス(Bacillus stearothermophilus) に属する９つの菌株と、サーマス(Thermus sp.) 属に属する２つの菌株からなる菌体混合物を用いた汚泥の消化（Shigeru KUME, et al., "DIGESTION OF MUNICIPAL SEWAGE SLUDGE BY A MIXTURE OF THERMOPHILIC BACILLI AND THEIR CULTURE EXTRACT", J. Gen. Appl. Microbiol., 36, 189-194 (1990)）；および滅菌済余剰汚泥を嫌気的条件下で特異的に可溶化する、Clostridium bifermentansに属する嫌気性の菌株（「バイオテクノロジーを活用した新排水処理システムの開発報告書（下水道編）」、pp.73-77、（財）土木研究センター（平成３年２月））、などがある。

【０００４】しかしながら、上記した特開平７－184640号の方法によれば、分解処理できる対象が実質的に酵母エキス残渣に限定されてしまい、また、KUMEらの方法ならびに土木研究センターによる菌株では、汚泥の消化処理（可溶化）効率が、それぞれ、10日間で25％、20日間で40～50％という低さであり、この処理効率の低さが当該菌株の工業的利用に向けての課題として依然として残っている。

【０００５】また、これまでに、有機性／生物性汚泥の固形分を効率的に可溶化する性能を備えた最近の分離・精製、および種の同定に至る報告は行われていなかった。

【０００６】

【課題を解決するための手段】本発明者らは、上述した従来技術での背景に鑑みて、有機性／生物性汚泥の固形分を効率的に可溶化する性能を備えた微生物を検索すべく鋭意検討を重ねた結果、好気高温消化槽より採取した消化汚泥から、所望の特性を有した細菌を単離し、その種まで同定するに至り、本願発明を完成ならしめたのである。

【０００７】すなわち、本願発明の要旨とするところは、有機性／生物性汚泥の固形分を効率的に可溶化する性能を有するバチルス・ステアロサーモフィラス(Bacillus stearothermophilus) に属する新規微生物である。

【０００８】本願発明によって取得された新規微生物の生物学的特徴を決定するために、この微生物が有する菌学的性質、すなわち、形態的性質、培地における生育状態、および生理学的性質に関して検定を行った。　その結果を以下にまとめた。

【０００９】本願新規微生物の菌学的性質

A．形態的性質

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(1) 細胞の形および大きさ：標準ブイヨンで、65℃、48時間培養したところ、 2.5～ 3.5× 0.3～ 0.5μのグラム陽性の直桿菌であり、短連鎖、束状に集合するものが認められた。 同様に、標準寒天培地で、培養後室温に４日間放置した場合、菌の形態に大きな変化が認められ、長い連鎖状のものが多くなり、湾曲したＳ字状のものや、一端に芽胞の形成が認められるもの、細胞内に顆粒が認められるものなどがあり、殆どがグラム陰性に染色された。

【００１０】(2) 運動性の有無：運動性は懸滴標本、半流動培地で確認できたが、周毛性の運動であり、比較的緩やかな滑走運動であった。

【００１１】(3) 胞子の有無：芽胞を形成し、形は卵円形で菌体より膨張している。 位置は端立であるが、形成初期には準端立に認められるものもある。 オタマジャクシ様のものも認められた。

【００１２】(4) グラム染色：細胞の多形性は著しく、特に、培養温度の影響を受けるのか、至適温度の60～65℃で正常な形態を示した。 グラム染色性も不定となる。

【００１３】Ｂ．培地における生育状態

Ｃ．生理学的性質

(1) グラム染色性：　＋
(2) 硝酸塩の還元能：　＋
(3) 脱窒反応〔硝酸塩ブイヨンでのガス産生〕：　＋
(4) ＭＲテスト：　＋
(5) ＶＰテスト：　－
(6) インドールの生成：　－
(7) 硫化水素の生成：　－
(8) デンプンの加水分解：　＋
(9) クエン酸の利用〔Simmns培地,Christensen培地共〕：　－
(10)無機窒素源の利用〔硝酸塩、アンモニウム塩〕：　＋
(11)色素の生成：　－（非水溶性）
(12)ウレアーゼ：　－
(13)オキシダーゼ：　＋
(14)カタラーゼ：　＋
(15)生育の範囲：　温度；　45～70℃／　ｐＨ；　5.0～ 7.0
(16)酸素に対する態度：　好気性～通性嫌気性
(17)Ｏ－Ｆ試験〔Hugh leifson法〕：　フラクトース（＋）
(18)糖類の利用と酸およびガス生成：

【００１８】　【表２】

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(1) 標準寒天平板培養：65℃、48時間の培養での集落の形状は、 0.5～１mmのスムーズな半球形の正円で光沢があるが、やや不透明な集落で一部周辺に膜状に僅かに拡散しているものも認められる。 しかし、一部のバチルス(Bacillus)属に属する細菌に認められる被膜状の拡散集落ではない。 また、色素の産生も認められなかった。

【００１４】(2) 標準ブイヨン培養：65℃、48時間の培養では、ブイヨンは一様に混濁し、発育良好である。しかし、培地表面での菌膜の形成等は認められなかった。 長時間室温にて放置すると、管底に雲恕状の沈殿を生じた。

【００１５】(3) 標準ゼラチン穿刺培養：培養して72時間後に、ゼラチン表層５mm、水平に液化した。 １週間後に、氷室に静置してもゼラチンは凝固しなくなった。

【００１６】(4) リトマス・ミルク：培地色は酸性化したが、凝固は認められなかった。 培養して72時間後に、表層より液化が始まり、１週間後に培地の1/3 が透明となった。

【００１７】

糖	酸			ガス	
	24時間	48時間	7日	48時間	7日
L－アラビノース	－	－	－	－	－
D－キシロース	－	－	－	－	－
D－グルコース	＋			－	－
D－マンノース	－	＋		－	－
D－フラクトース	－	－	－	－	－
D－ガラクトース	－	－	－	－	－
マルトース	－	＋		－	－
シュークロース	－	＋		－	－
ラクトース	－	－	－	－	－
トレハロース	－	＋		－	－
D－ソルビトール	－	－	－	－	－
D－マンニトール	－	＋		－	－
イノシトール	－	－	－	－	－
グリセリン	－	＋		－	－
デンプン	－	－	－	－	－

【００１９】上記した新規微生物の諸性質は、バチルス・ステアロサーモフィラス（Bacillus stearothermophilus)が有する諸性質とよく対応したので、バチルス・ステアロサーモフィラス SPT2-1 (Bacillus stearothermophilus SPT2-1)と命名した。なお、本願発明にて得られた、バチルス・ステアロサーモフィラス SPT2-1(Bacillus stearothermophilus SPT2-1)は、平成８年１月18日に、本願出願人によって、茨城県つくば市東町１丁目１番３号に所在の通商産業省工業技術院生命工学工業技術研究所にて寄託され、そして、受託番号 FERM P-15395 が付与されており、本願発明は、この寄託微生物自体はもちろん、前述した能力を有するその変異体および子孫まで意図するものである。

【００２０】本発明のその他の態様および利点は、以下に例示的に示した実施例の開示から明らかである。

【００２１】

【実施例】

実施例１：バチルス・ステアロサーモフィラス SPT2-1の単離

３ヶ月連続して運転した好気高温消化槽（70℃）での消化汚泥より、菌株分離用に汚泥を採取した。

【００２２】採取した汚泥を、1×10^{-3}～10^{-5}倍に滅菌水で適当に希釈した後、ＹＰ寒天プレート(DIFCO社製)に塗布した。　このプレートを、65℃で一晩培養し、単コロニーを得た。

【００２３】得られた単コロニーに関して、デンプンの分解能、スキムミルクの分解能、滅菌汚泥の分解能を、基質混合培地でのハロ形成の有無で確認し、ハロを形成した株でも、特に汚泥分解能が大きいものを特徴株として選抜した。　なお、デンプンおよびスキムミルクの分解能の検定は、R. BEAUDET, C. GAGNON, J.G. BISAILLON and M. ISHAQUE, "Microbiological Aspects of Aerobic Thermophilic Treatment of Swine Waste", Applied and Environmental Microbiology, pp.971-976, April 1990の変法によった。

【００２４】これら特徴株について、滅菌汚泥の分解活性をさらに検討し、最も活性の高い株をSPT2-1株と命名した。　また、同様に滅菌汚泥の分解活性を示した株(SPT1-6)を、比較用の株として選抜した。

【００２５】実施例２：SPT2-1株の活性に対するpHおよび温度が与える影響

0.1N塩酸（和光純薬社製）および／または0.1N水酸化ナトリウム（和光純薬社製）で、測定対象のpH（pH 4.8、5.0、5.2、5.5、5.7、6.8、7.0）に調整したYP培地(DIFCO社製：イーストエキス４ｇ、ペプトン８ｇ、水１ｌ；pH6.8)を調製し、この培地に菌株（SPT2-1株）を接種し、70℃で６時間培養した SPT2-1株の増殖菌体量を観察し、その生育度を定性的に測定した。　その結果を、下記表３に示した。

【００２６】

【表３】

pH	生育度
4.8	－
5.0	++
5.2	++
5.5	++
5.7	++
6.8	++
7.0	＋

凡例：　生育度（－）：生育せず
（＋）：通常の生育
（++）：活発な生育

【００２７】表３の結果から、SPT2-1株が、pH 5.0～6.8の範囲のpHにおいて活発に生育することが判明した。

【００２８】一方で、0.1N塩酸（和光純薬社製）および／または0.1N水酸化ナトリウム（和光純薬社製）で、pH 6.8に調整したYP培地(DIFCO社製：イーストエキス４

ｇ、ペプトン８ｇ、水１１；pH6.8)を調製し、この培地に菌株（SPT2-1株）を接種し、測定対象の温度（10、25、45、55、60、65、70および80℃）で、６時間培養したSPT2-1株の増殖菌体量を観察し、その生育度を定性的に測定した。　その結果を、下記表４に示した。

【００２９】

【表４】

温度	生育度
10	－
25	－
45	±
55	＋
60	＋＋
65	＋＋
70	＋＋
80	－

凡例：　生育度（－）：生育せず
（±）：わずかに生育
（＋）：通常の生育
（＋＋）：活発な生育

【００３０】表４の結果から、SPT2-1株が、45～70℃、特に、60～70℃の温度範囲において活発な生育状態を示すことが判明した。

【００３１】実施例３：汚泥可溶化効率の検定

供試菌株（一白金耳：SPT2-1株とSPT1-6株）を、YP培地(DIFCO社製：イーストエキス４ｇ、ペプトン８ｇ、水１１；pH6.8)の培地で、70℃で、15時間培養する。　培養した菌株を、１％（vol./vol.)の濃度になるように滅菌汚泥（初発ＶＭ（Volatile Matter:全有機物質）：11000ppm、ＶＳＳ：11000ppm）に接種して、70℃で振とう培養を行った。　振とう培養を終えて一定時間（24時間および48時間）後に、試料採取を行い、試料中の有機性固形分（ＶＳＳ：Volatile Suspended Solids)の測定を行い、以下の関係式に基づいて、固形分の減少量から可溶化率を求めた。

【００３２】

【数１】

$$\text{ＶＳＳ可溶化率（％）} = \frac{\text{初発ＶＳＳ} - \text{培養後ＶＳＳ}}{\text{初発ＶＳＳ}} \times 100$$

【００３３】その結果を、下記表５と図１に示した。なお、滅菌汚泥は、「バイオテクノロジーを活用した新排水処理システムの開発報告書（下水道編）」、第73頁～第77頁、（財）土木研究センター（平成３年２月）の記載に従って調製した。　また、ＶＳＳの測定は、下水試験法（1984年版）に準じて行った。

【００３４】

【表５】

時間（日）		ＶＳＳ可溶化率（％） 対照	SPT2-1	SPT1-6
0日	1回目	1	2.5	1
	2回目	－1	－2.5	－1
	平均	0	0	0
1日	1回目	10.4	18.8	15.2
	2回目	4.7	21.9	13.8
	平均	8	20.3	14.5
2日	1回目	7.4	38.1	10.4
	2回目	7.4	33.9	14.2
	平均	7.4	36	12.7

【００３５】実施例４：熱難分解性デンプン含有排水（デンプン工場排水）の可溶化効率の検定

供試菌株を、YP培地(DIFCO社製：イーストエキス４ｇ、ペプトン８ｇ、水１１；pH6.8)の培地で、70℃で、15時間培養する。　培養した菌株を、１％（vol./vol.)の濃度になるように、熱難分解性デンプン含有排水（デンプン工場排水、初発ＶＭ：10,000ppm、ＶＳＳ：7,000ppm）に接種して、70℃で、24時間、48時間および72時間振とう培養を行った。　各々の培養時間後に試料採取を行い、試料中の有機性固形分（ＶＳＳ）の測定を行い（前掲の下水試験法（1984年版）に従った）、以下の関係式に基づいて、固形分の減少量から可溶化率を求めた。

【００３６】

【数２】

$$\text{ＶＳＳ可溶化率（％）} = \frac{\text{初発ＶＳＳ} - \text{培養後ＶＳＳ}}{\text{初発ＶＳＳ}} \times 100$$

【００３７】その結果を、下記表６と図２に示した。

【００３８】

【表６】

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時　間（日）	ＶＳＳ可溶化率（%）		
	対　照	SPT2-1	SPT1-6
0日	0	0	0
1日	0.1	33.6	14.7
2日	-0.8	36.2	24.7
3日	0.2	50.4	36.8

【００３９】

【発明の効果】本発明により、有機性汚泥あるいは生物性汚泥の固形分、特に、余剰汚泥およびデンプン廃液等を特異的に効率良く可溶化する性能を有する、バチルス・ステアロサーモフィラス(Bacillus stearothermophilus）に属する新規微生物、バチルス・ステアロサーモフィラスSPT2-1（Bacillus stearothermophilus SPT2-1）が提供されるのである。　すなわち、本発明によって提供された新規微生物は、有機性汚泥あるいは生物性汚泥の固形分を効率よく可溶化するなどの効果を奏する。

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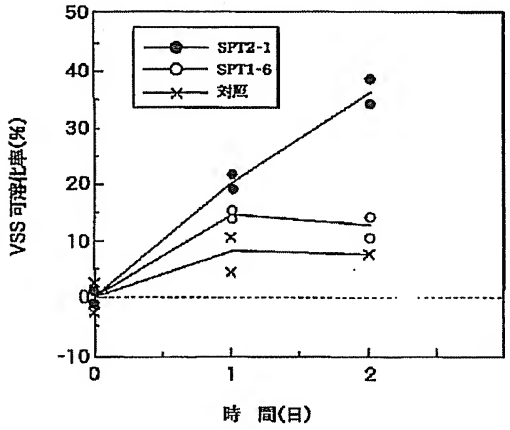
【図面の簡単な説明】

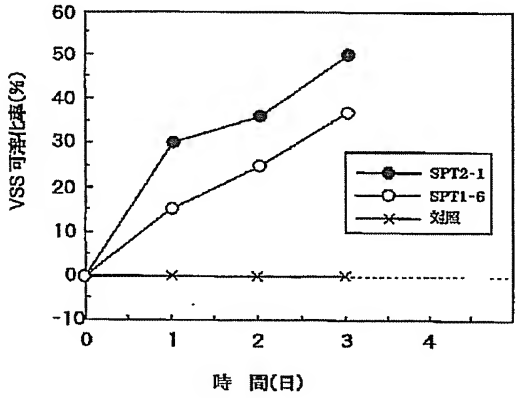
【図１】　バチルス・ステアロサーモフィラス SPT2-1の滅菌汚泥の可溶化効率の検定結果を示すグラフである。

【図２】　バチルス・ステアロサーモフィラス SPT2-1の熱難分解性デンプン含有排水固形分の可溶化効率の検定結果を示すグラフである。

【図１】



【図２】



【手続補正書】

【提出日】平成８年４月５日

【手続補正１】

【補正対象書類名】明細書

【補正対象項目名】請求項４

【補正方法】変更

【補正内容】

【請求項４】　前記バチルス・ステアロサーモフィラスに属する新規微生物が、以下の菌学的性質、すなわち；

Ａ．形態的性質	
(1) 細胞の形：	桿菌
(2) 運動性の有無：	＋
(3) 胞子の有無：	＋
(4) グラム染色：	＋
Ｂ．培地における生育状態	
(1) 普通寒天平板培養：	＋
(2) 普通ブイヨン培養：	＋
(3) ゼラチン穿刺培養：	凝集しない
(4) リトマス・ミルク：	＋
Ｃ．生理学的性質	
(1) グラム染色性：	＋
(2) 硝酸塩の還元能：	＋
(3) 脱窒反応〔硝酸塩ブイヨンでのガス産生〕：	＋
(4) ＭＲテスト：	＋
(5) ＶＰテスト：	－

(6) インドールの生成：　－
(7) 硫化水素の生成：　－
(8) デンプンの加水分解：　＋
(9) クエン酸の利用〔Simmns培地,Christensen培地共〕：　－
(10)無機窒素源の利用〔硝酸塩、アンモニウム塩〕：　＋
(11)色素の生成：　－（非水溶性）
(12)ウレアーゼ：　－
(13)オキシダーゼ：　＋
(14)カタラーゼ：　＋
(15)生育の範囲：　温度；　45～70℃／　ｐＨ；　5.0～ 7.0
(16)酸素に対する態度：　好気性～通性嫌気性
(17)Ｏ－Ｆ試験〔Hugh leifson法〕：　Ｆ（発酵）
(18)糖類の利用と酸およびガス生成：

【表１】

糖	酸			ガス	
	24時間	48時間	7日	48時間	7日
Ｌ－アラビノース	－	－	－	－	－
Ｄ－キシロース	－	－	－		－
Ｄ－グルコース	＋			－	－
Ｄ－マンノース	－	＋		－	－
Ｄ－フラクトース	－	－	－	－	－
Ｄ－ガラクトース	－	－	－	－	－
マルトース	－	＋		－	－
シュークロース	－	＋		－	－
ラクトース	－	－	－	－	
トレハロース	－	＋		－	－
Ｄ－ソルビトール	－	－	－	－	－
Ｄ－マンニトール	－	＋		－	－
イノシトール	－	－	－	－	－
グリセリン	－	＋		－	－
デンプン	－	－	－	－	－

の菌学的性質を有する請求項１に記載の新規微生物。
【手続補正２】
【補正対象書類名】明細書
【補正対象項目名】０００９
【補正方法】変更
【補正内容】
【０００９】本願新規微生物の菌学的性質
Ａ．形態的性質
(1) 細胞の形および大きさ：普通ブイヨンで、65℃、48時間培養したところ、 2.5～ 3.5× 0.3～ 0.5μのグラム陽性の直桿菌であり、短連鎖、束状に集合するものが認められた。　同様に、普通寒天培地で、培養後室温に４日間放置した場合、菌の形態に大きな変化が認められ、長い連鎖状のものが多くなり、湾曲したＳ字状のものや、一端に芽胞の形成が認められるもの、細胞内に顆粒が認められるものなどがあり、殆どがグラム陰性に染色された。
【手続補正３】
【補正対象書類名】明細書
【補正対象項目名】００１３
【補正方法】変更
【補正内容】
【００１３】Ｂ．培地における生育状態
(1) 普通寒天平板培養：65℃、48時間の培養での集落の形状は、 0.5～１㎜のスムーズな半球形の正円で光沢があるが、やや不透明な集落で一部周辺に膜状に僅かに拡散しているものも認められる。　しかし、一部のバチルス(Bacillus)属に属する細菌に認められる被膜状の拡散集落ではない。　また、色素の産生も認められなかった。
【手続補正４】
【補正対象書類名】明細書
【補正対象項目名】００１４
【補正方法】変更
【補正内容】
【００１４】(2) 普通ブイヨン培養：65℃、48時間の培養では、ブイヨンは一様に混濁し、発育良好である。しかし、培地表面での菌膜の形成等は認められなかった。　長時間室温にて放置すると、管底に雲恕状の沈殿を生じた。

【手続補正５】
【補正対象書類名】明細書
【補正対象項目名】００１５
【補正方法】変更
【補正内容】
【００１５】(3) ゼラチン穿刺培養：培養して72時間後に、ゼラチン表層５㎜、水平に液化した。　１週間後に、氷室に静置してもゼラチンは凝固しなくなった。

【手続補正６】
【補正対象書類名】明細書
【補正対象項目名】００１７
【補正方法】変更
【補正内容】
【００１７】

C．生理学的性質

(1) グラム染色性：	＋
(2) 硝酸塩の還元能：	＋
(3) 脱窒反応〔硝酸塩ブイヨンでのガス産生〕：	＋
(4) ＭＲテスト：	＋
(5) ＶＰテスト：	－
(6) インドールの生成：	－
(7) 硫化水素の生成：	－
(8) デンプンの加水分解：	＋
(9) クエン酸の利用〔Simmns培地,Christensen培地共〕：	－
(10)無機窒素源の利用〔硝酸塩、アンモニウム塩〕：	＋
(11)色素の生成：	－（非水溶性）
(12)ウレアーゼ：	－
(13)オキシダーゼ：	＋
(14)カタラーゼ：	＋
(15)生育の範囲：　温度；　45～70℃／　ｐＨ；　5.0～ 7.0	
(16)酸素に対する態度：　好気性～通性嫌気性	
(17)Ｏ－Ｆ試験〔Hugh leifson法〕：　Ｆ（発酵）	
(18)糖類の利用と酸およびガス生成：	

フロントページの続き

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(54) NEW MICROORGANISM

(57)Abstract:
PROBLEM TO BE SOLVED: To specifically solubilize sewage sludge, starch waste fluid, etc., by acting a new microorganism capable of solubilizing a solid component of an organic/biological sludge thereon.
SOLUTION: A new microorganism belonging to the genus Bacillus stearothermophilus capable of solubilizing a solid component of an organic/ biological sludge, e.g. Bacillus stearothermophilus SPT2-1 (FERM P-15395) which is collected from a high temperature aerobic digestion vessel.

LEGAL STATUS

[Date of request for examination]	10.10.2000
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[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
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[Number of appeal against examiner's decision of rejection]	2003-22834
[Date of requesting appeal against examiner's decision of rejection]	25.11.2003
[Date of extinction of right]	

CLAIMS

[Claim(s)]
[Claim 1] Bacillus stearothermophilus which has the engine performance which solubilizes the solid content of organic sludge or living thing nature sludge (Bacillus stearothermophilus) New microorganism which belongs.
[Claim 2] The new microorganism according to claim 1 said organic sludge or whose living thing nature sludge is sewage excess sludge.
[Claim 3] The new microorganism according to claim 1 said whose organic sludge is starch waste fluid.
[Claim 4] the new microorganism belonging to said Bacillus stearothermophilus -- the following mycology-properties, i.e.,;
A. Gestalt-property (1) Form of a cell : ** ** bacillus (2) Motile existence : + (3) Existence of a spore : + (4) Gram's stain : Growth condition in a ** B. culture medium (1) Standard agar plate culture : + (2) Standard bouillon culture: + (3) Standard gelatin stab culture : - (4) Litmus milk: + C. physiological property (1) Gram's stain nature : + (2) Reduction ability of a nitrate : + (3) Aerosis [in denitrification reaction [nitrate bouillon]]: + (4) MR test: + (5) VP test : - (6) Generation of Indore : - (7) Generation of a hydrogen sulfide : - (8) Hydrolysis of starch : + (9) Use of a citric acid [a Simmns culture medium and a Christensen culture medium]: - Use of the source of (10) inorganic nitrogen [a nitrate and ammonium salt]: + Generation of (11) coloring matter: - (nonaqueous solubility)
(12) Urease : - (13) oxidases : + (14) catalases : + Range of (15) growth: Temperature; 45-70degree-C/ pH; 5.0-7.0 Attitude against (16) oxygen: aerotropism - a denominator -- anaerobiosis (17) O-F trial [Hugh leifson -- law]: -- fructose (+)
(18) Use and the acid of a saccharide, and gas generation : [Table 1]

	酸発			ガス	
糖類	24時間	48時間	7日	48時間	7日
L-アラビノース	-	-	-	-	-
D-キシロース	-	-	-	-	-
D-グルコース	+			-	-
D-マンノース	-	+		-	-
D-フラクトース	-	-	-	-	-
D-ガラクトース	-	-	-	-	-
マルトース	-	+		-	-
シュークロース	-	+		-	-
ラクトース	-	-	-	-	-
トレハロース	-	+		-	-
D-ソルビトール	-	-	-	-	-
D-マンニトール	-	+		-	-
イノシトール	-	-	-	-	-
グリセリン	-	+		-	-
デンプン	-	-	-	-	-

The new microorganism according to claim 1 which has a ******-property.
[Claim 5] the new microorganism belonging to said Bacillus stearothermophilus -- Bacillus stearothermophilus (Bacillus stearothermophilus) [FERM P-15395] SPT 2-1 it is -- new microorganism according to claim 4.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the new microorganism equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge.
[0002]
[Description of the Prior Art] The strain it is expected that the application to the approach and this approach for solubilization of the following organic sludge of making bacteria etc. act on sludge and carrying out decomposition processing of the sludge biologically as an art of the organic nature high concentration sludge conventionally discharged from the living thing nature sludge discharged from places of business, such as a sewage disposal plant and a human waste treatment plant, or food works is is reported until now.
[0003] For example, the strain which produces the enzyme which decomposes yeast extract residue specifically, The bacteria belonging to an OERUSU Coffea group () [Oerskovia] sp.24: (FERM P-13692) Refer to Japanese Patent Application No. No. 211081 [five to]. Art of the used yeast extract residue (refer to JP,7-184640,A); Isolated from sludge compost under the high temperature service aerobically. Bacillus stearothermophilus which has the optimum growth temperature of 65 degrees C (Bacillus stearothermophilus) Nine strain which belongs, Thermus (Thermus sp.) () Digestion of the sludge using the fungus body mixture which consists of two strain belonging to a group [Shigeru KUME, et al.,] ["DIGESTION OF MUNICIPAL SEWAGE] SLUDGE BY A MIXTURE OF THERMOPHILIC BACILLI AND THEIR CULTURE EXTRACT", J.Gen.Appl.Microbiol., 36, 189-194; (1990), and sterilized excess sludge the anaerobic strain ("the development report (volume on sewerage) of the new waste-water-treatment system which utilized biotechnology" --) belonging to Clostridium bifermentans specifically solubilized under an anaerobic condition There are pp.73-77, Public Works Research Center (February, Heisei 3), etc.
[0004] However, according to the above-mentioned approach of JP,7-184640,A The object which can carry out decomposition processing will be substantially limited to yeast extract residue, and in KUME's and others approach, and the strain by Public Works Research Center The digestive treatment (solubilization) effectiveness of sludge is the lowness which calls 25% in ten days and it calls 40 - 50% in 20 days, respectively, and it still remains as a technical problem which the lowness of this processing effectiveness turns to industrial use of the strain concerned.
[0005] Moreover, latest separation and purification equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge until now, and the report which results in identification of a seed were not performed.
[0006]
[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge should be searched in view of the background in the conventional technique mentioned above, from the digested sludge extracted from the aerobic thermophilic-digestion tub, this invention persons isolate bacteria with a desired property, came to identify even the kind, and if they were completion, they closed the invention in this application.
[0007] That is, the place made into the summary of the invention in this application is Bacillus stearothermophilus (Bacillus stearothermophilus) which has the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge. It is the new microorganism which belongs.
[0008] In order to determine the biological description of the new microorganism acquired by the invention in this application, it authorized about the mycology-property which this microorganism has, i.e., a gestalt-property, the growth condition in a culture medium, and the physiological property. The result was summarized to below.
[0009] Mycology-property A. gestalt-property of this application new microorganism (1) A form and magnitude of a cell: With standard bouillon, it is the gram-positive direct Bacillus of 2.5- 3.5x 0.3- 0.5micro, and 65 degrees C of things which gather the short chain and in the shape of a bundle were accepted, when cultivated for 48 hours. Similarly, by the standard agar medium, when it was left for four days in the room temperature after culture, a big change is accepted in the gestalt of a bacillus, the thing of the shape of a long chain increases, there are a thing of the shape of S curved character, that formation of a spore is accepted to be to an end, the thing granulation is accepted to be to intracellular, etc., and most was dyed gram-negative.
[0010] (2) Motile existence : although maneuverability has been checked by hanging drop preparation and the semi solid medium, it was peritrichate movement and was comparatively loose gliding motility.
[0011] (3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape. Although a location is ****, it has some which are accepted in semi- **** in early stages of formation. The tadpole's thing was also accepted.
[0012] (4) Gram's stain : the polymorphism of a cell was remarkable and the gestalt of optimum temperature normal at 60-65 degrees C was shown for whether it is especially influenced of culture temperature. Gram's stain nature also serves as an indeterminate.
[0013] B. Growth condition in a culture medium (1) Standard agar plate culture: Although it is glossy with the configuration of the cluster in culture of 65 degrees C and 48 hours, and a 0.5-1mm semi-sphere smooth right circle, what is slightly diffused in the shape of film on the outskirts in part in a little opaque cluster is accepted. However, it is not the diffusion cluster of the shape of a coat accepted in the bacteria belonging to some bacillus (Bacillus) groups. Moreover, production of coloring matter was not accepted, either.
[0014] (2) Standard bouillon culture : in 65 degrees C and culture of 48 hours, bouillon becomes muddy uniformly, and growth is good. However, formation of the pellicle on the front face of a culture medium etc. was not accepted. When it was left at the long duration room temperature, ****-like precipitate was produced in the tube bottom.
[0015] (3) Standard gelatin stab culture : 72 hours after cultivating, it liquefied horizontally 5mm of gelatin surfaces. Even if it put on Himuro gently after one week, it stopped solidifying gelatin.
[0016] (4) Litmus milk : coagulation was not accepted although the culture-medium color acidified. 72 hours after cultivating, liquefaction starts from a surface, and it is one third of culture media after one week. It became transparence.
[0017]
C. Physiological property (1) Gram's stain nature : + (2) Reduction ability of a nitrate : + (3) Aerosis [in denitrification reaction

[nitrate bouillon]]: + (4) MR test : + (5) VP test : - Generation of (6) Indore : - (7) Generation of a hydrogen sulfide : - (8) Hydrolysis of starch : + (9) Use of a citric acid [a Simmns culture medium and a Christensen culture medium]: Use of the source of -(10) inorganic nitrogen [a nitrate and ammonium salt]: Generation of + (11) coloring matter: - (nonaqueous solubility) (12) Urease : - (13) oxidases : + (14) catalases : + range of (15) growth: Temperature; 45-70degree-C/ pH; Attitude against 5.0-7.0 (16) oxygen: aerotropism - a denominator -- anaerobic (17) O-F trial [Hugh leifson -- law]: Fructose (+) (18) Use and the acid of a saccharide, and gas generation : [0018]

[Table 2]

糖	酸			ガス	
	24時間	48時間	7日	48時間	7日
L-アラビノース	-	-	-	-	-
D-キシロース	-	-	-	-	-
D-グルコース	+			-	-
D-マンノース	-	+		-	-
D-フラクトース	-	-	-	-	-
D-ガラクトース	-	-	-	-	-
マルトース	-	+		-	-
シュークロース	-	+		-	-
ラクトース	-	-	-	-	-
トレハロース	-	+		-	-
D-ソルビトール	-	-	-	-	-
D-マンニトール	-	+		-	-
イノシトール	-	-	-	-	-
グリセリン	-	+		-	-
デンプン	-	-	-	-	-

[0019] Many properties of the above-mentioned new microorganism are Bacillus stearothermophilus. (Bacillus stearothermophilus) Since it corresponded well with many properties which it has, it is Bacillus stearothermophilus. It was named SPT 2-1 (Bacillus stearothermophilus SPT 2-1). In addition, Bacillus stearothermophilus obtained in the invention in this application An applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on January 18, Heisei 8, and SPT 2-1 (Bacillus stearothermophilus SPT 2-1) is a trust number. FERM P-15395 It is given and even that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself mean the invention in this application.

[0020] Other modes and advantages of this invention are clear to below from the indication of the example shown in instantiation.

[0021]

[Example]

Example 1: Bacillus stearothermophilus SPT 2-1 From the digested sludge in the aerobic thermophilic-digestion tub (70 degrees C) continuously operated for isolation three months, sludge was extracted to strain separation.

[0022] After diluting the extracted sludge with sterilized water suitably 1x10-3 to 10 to 5 times, it was applied to YP agar plate (product made from DIFCO). This plate was cultivated at 65 degrees C overnight, and the single colony was obtained.

[0023] the stock which checked the resolution of starch, the resolution of skim milk, and the resolution of sterilization sludge by the existence of the halo formation by the substrate mixing culture medium, and formed the halo about the obtained single colony -- especially -- sludge -- what has large resolution was selected as a description stock. In addition, assay of the resolution of starch and skim milk was based on the strange method of R.BEAUDET, C.GAGNON, J.G.BISAILLON and M.ISHAQUE, "Microbiological Aspects of Aerobic Thermophilic Treatment of Swine Waste", Applied and Environmental Microbiology, pp.971-976, and April 1990.

[0024] About these descriptions stock, the decomposition activity of sterilization sludge was examined further, and the stock with the highest activity was named two to one share of SPT. Moreover, the stock (SPT 1-6) in which the decomposition activity of sterilization sludge was shown similarly was selected as a stock for a comparison.

[0025] By 0.1N hydrochloric acid (Wako Pure Chem make) of Example 2:effects which pH and temperature over the activity of two to one share of SPT have, and/or 0.1-N sodium hydroxide (Wako Pure Chem make) YP culture medium (: made from DIFCO -- yeast extractives 4g --) adjusted to pH (pH 4.8, 5.0, 5.2, 5.5, 5.7, 6.8, 7.0) of the measuring object Peptone 8g and water 1 l;pH6.8 were prepared, strain (two to one share of SPT) was inoculated into this culture medium, and it cultivated at 70 degrees C for 6 hours. The growth cell mass of two to one share of SPT was observed, and whenever [that growth] was measured qualitatively. The result was shown in the following table 3.

[0026]

[Table 3]

pH	生育度
4.8	−
5.0	++
5.2	++
5.5	++
5.7	++
6.8	++
7.0	+

凡例： 生育度（−）：生育せず
（+）：通常の生育
（++）：活発な生育

[0027] From the result of Table 3, it became clear that two to one share of SPT grew actively in pH of the range of pH 5.0- 6.8.

[0028] By on the other hand, 0.1-N hydrochloric acid (Wako Pure Chem make) and/or 0.1-N sodium hydroxide (Wako Pure Chem make) YP culture medium (: made from DIFCO — yeast extractives 4g —) adjusted to pH 6.8 pH6.8 was prepared, strain (two to one share of SPT) was inoculated into this culture medium, the growth cell mass of two to one share of peptone 8g and 1l. [of water]; SPT cultivated for 6 hours at the temperature (10, 25, 45, 55, 60, 65, 70, and 80 degrees C) of the measuring object was observed, and whenever [that growth] was measured qualitatively. The result was shown in the following table 4.
[0029]
[Table 4]

温度	生育度
10	−
25	−
45	±
55	+
60	++
65	++
70	++
80	−

凡例： 生育度（−）：生育せず
（±）：わずかに生育
（+）：通常の生育
（++）：活発な生育

[0030] From the result of Table 4, it became clear that two to one share of SPT showed especially 45-70 degrees C of active growth conditions in a 60-70-degree C temperature requirement.
[0031] Example 3: Cultivate the assay sample offering strain (one platinum loop: two to one share of SPT, and one to six shares of SPT) of sludge solubilization effectiveness at 70 degrees C for 15 hours by the culture medium of YP culture medium (the product made from DIFCO: yeast extractives 4g, peptone 8g, water 1 l;pH6.8). The cultivated strain was inoculated into sterilization sludge (initiation VM(Volatile Matter: all organic substances):11000ppm, VSS:11000ppm) so that it might become 1% (vol./vol.) of concentration, and shaking culture was performed at 70 degrees C. Shaking culture was finished, after fixed time amount (24 hours and 48 hours), sampling was performed, organic nature solid content in a sample (VSS:Volatile Suspended Solids) was measured, and the rate of solubilization was calculated from the decrement of solid content based on the following relational expression.
[0032]
[Equation 1]

$$\text{VSS可溶化率（\%）} = \frac{\text{初発VSS} - \text{培養後VSS}}{\text{初発VSS}} \times 100$$

[0033] The result was shown in the following table 5 and drawing 1 . In addition, sterilization sludge was prepared according to the publication of "the development report (volume on sewerage) of the new waste-water-treatment system which utilized biotechnology", 73rd page - the 77th page, and Public Works Research Center (February, Heisei 3). Moreover, measurement of VSS was performed according to the test of sewage (1984 editions).
[0034]
[Table 5]

時間（日）		ＶＳＳ可溶化率（%） 対照	SPT2-1	SPT1-6
0日	1回目	1	2.5	1
	2回目	−1	−2.5	−1
	平均	0	0	0
1日	1回目	10.4	18.8	15.2
	2回目	4.7	21.9	13.8
	平均	8	20.3	14.5
2日	1回目	7.4	38.1	10.4
	2回目	7.4	33.9	14.2
	平均	7.4	36	12.7

[0035] Example 4: Cultivate the assay sample offering strain of the solubilization effectiveness of **** resolvability starch content wastewater (starch industrial liquid waste) at 70 degrees C for 15 hours by the culture medium of YP culture medium (the product made from DIFCO: yeast extractives 4g, peptone 8g, water 1 l;pH6.8). The cultivated strain was inoculated into **** resolvability starch content wastewater (starch industrial liquid waste, initiation VM:10,000ppm, VSS:7,000ppm) so that it might become 1% (vol./vol.) of concentration, and shaking culture was performed at 70 degrees C for 24 hours, 48 hours, and 72 hours. Sampling was performed after each culture time amount, organic nature solid content (VSS) in a sample was measured (the test of sewage (1984 editions) shown above was followed), and the rate of solubilization was calculated from the decrement of solid content based on the following relational expression.

[0036]

[Equation 2]

$$\text{ＶＳＳ可溶化率（%）} = \frac{\text{初発ＶＳＳ} - \text{培養後ＶＳＳ}}{\text{初発ＶＳＳ}} \times 100$$

[0037] The result was shown in the following table 6 and drawing 2 .

[0038]

[Table 6]

時間（日）	ＶＳＳ可溶化率（%） 対照	SPT2-1	SPT1-6
0日	0	0	0
1日	0.1	33.6	14.7
2日	-0.8	36.2	24.7
3日	0.2	50.4	36.8

[0039]

[Effect of the Invention] Bacillus stearothermophilus which has the solid content of organic sludge or living thing nature sludge, and the engine performance which solubilizes excess sludge, starch waste fluid, etc. efficiently specifically especially by this invention (Bacillus stearothermophilus) The new microorganism and Bacillus stearothermophilus SPT 2-1 (Bacillus stearothermophilus SPT 2-1) which belong are offered. That is, the new microorganism offered by this invention does so the effectiveness of solubilizing efficiently the solid content of organic sludge or living thing nature sludge.

[Translation done.]

TECHNICAL FIELD

[Industrial Application] This invention relates to the new microorganism equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention] Bacillus stearothermophilus which has the solid content of organic sludge or living thing nature sludge, and the engine performance which solubilizes excess sludge, starch waste fluid, etc. efficiently specifically especially by this invention (Bacillus stearothermophilus) The new microorganism and Bacillus stearothermophilus SPT 2-1 (Bacillus stearothermophilus SPT 2-1) which belong are offered. That is, the new microorganism offered by this invention does so the effectiveness of solubilizing efficiently the solid content of organic sludge or living thing nature sludge.

[Translation done.]

TECHNICAL PROBLEM

[Description of the Prior Art] The strain it is expected that the application to the approach and this approach for solubilization of the following organic sludge of making bacteria etc. act on sludge and carrying out decomposition processing of the sludge biologically as an art of the organic nature high concentration sludge conventionally discharged from the living thing nature sludge discharged from places of business, such as a sewage disposal plant and a human waste treatment plant, or food works is is reported until now.

[0003] For example, the strain which produces the enzyme which decomposes yeast extract residue specifically, The bacteria belonging to an OERUSU Coffea group () [Oerskovia] sp.24: (FERM P-13692) Refer to Japanese Patent Application No. No. 211081 [five to]. Art of the used yeast extract residue (refer to JP,7-184640,A); Isolated from sludge compost under the high temperature service aerobically. Bacillus stearothermophilus which has the optimum growth temperature of 65 degrees C (Bacillus stearothermophilus) Nine strain which belongs, Thermus (Thermus sp.) () Digestion of the sludge using the fungus body mixture which consists of two strain belonging to a group [Shigeru KUME, et al.,] ["DIGESTION OF MUNICIPAL SEWAGE] SLUDGE BY A MIXTURE OF THERMOPHILIC BACILLI AND THEIR CULTURE EXTRACT", J.Gen.Appl.Microbiol., 36, 189-194; (1990), and sterilized excess sludge the anaerobic strain ("the development report (volume on sewerage) of the new waste-water-treatment system which utilized biotechnology" --) belonging to Clostridium bifermentans specifically solubilized under an anaerobic condition There are pp.73-77, Public Works Research Center (February, Heisei 3), etc.

[0004] However, according to the above-mentioned approach of JP,7-184640,A The object which can carry out decomposition processing will be substantially limited to yeast extract residue, and in KUME's and others approach, and the strain by Public Works Research Center The digestive treatment (solubilization) effectiveness of sludge is the lowness which calls 25% in ten days and it calls 40 - 50% in 20 days, respectively, and it still remains as a technical problem which the lowness of this processing effectiveness turns to industrial use of the strain concerned.

[0005] Moreover, latest separation and purification equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge until now, and the report which results in identification of a seed were not performed.

[Translation done.]

MEANS

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the microorganism equipped with the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge should be searched in view of the background in the conventional technique mentioned above, from the digested sludge extracted from the aerobic thermophilic-digestion tub, this invention persons isolate bacteria with a desired property, came to identify even the kind, and if they were completion, they closed the invention in this application.
[0007] That is, the place made into the summary of the invention in this application is Bacillus stearothermophilus (Bacillus stearothermophilus) which has the engine performance which solubilizes efficiently the solid content of organic nature / living thing nature sludge. It is the new microorganism which belongs.
[0008] In order to determine the biological description of the new microorganism acquired by the invention in this application, it authorized about the mycology-property which this microorganism has, i.e., a gestalt-property, the growth condition in a culture medium, and the physiological property. The result was summarized to below.
[0009] Mycology-property A. gestalt-property of this application new microorganism (1) A form and magnitude of a cell: With standard bouillon, it is the gram-positive direct Bacillus of 2.5- 3.5x 0.3- 0.5micro, and 65 degrees C of things which gather the short chain and in the shape of a bundle were accepted, when cultivated for 48 hours. Similarly, by the standard agar medium, when it was left for four days in the room temperature after culture, a big change is accepted in the gestalt of a bacillus, the thing of the shape of a long chain increases, there are a thing of the shape of S curved character, that formation of a spore is accepted to be to an end, the thing granulation is accepted to be to intracellular, etc., and most was dyed gram-negative.
[0010] (2) Motile existence : although maneuverability has been checked by hanging drop preparation and the semi solid medium, it was peritrichate movement and was comparatively loose gliding motility.
[0011] (3) Existence of a spore : forming a spore, the form is expanding from the fungus body by the egg round shape. Although a location is ****, it has some which are accepted in semi- **** in early stages of formation. The tadpole's thing was also accepted.
[0012] (4) Gram's stain : the polymorphism of a cell was remarkable and the gestalt of optimum temperature normal at 60-65 degrees C was shown for whether it is especially influenced of culture temperature. Gram's stain nature also serves as an indeterminate.
[0013] B. Growth condition in a culture medium (1) Standard agar plate culture: Although it is glossy with the configuration of the cluster in culture of 65 degrees C and 48 hours, and a 0.5-1mm semi-sphere smooth right circle, what is slightly diffused in the shape of film on the outskirts in part in a little opaque cluster is accepted. However, it is not the diffusion cluster of the shape of a coat accepted in the bacteria belonging to some bacillus (Bacillus) groups. Moreover, production of coloring matter was not accepted, either.
[0014] (2) Standard bouillon culture : in 65 degrees C and culture of 48 hours, bouillon becomes muddy uniformly, and growth is good. However, formation of the pellicle on the front face of a culture medium etc. was not accepted. When it was left at the long duration room temperature, ****-like precipitate was produced in the tube bottom.
[0015] (3) Standard gelatin stab culture : 72 hours after cultivating, it liquefied horizontally 5mm of gelatin surfaces. Even if it put on Himuro gently after one week, it stopped solidifying gelatin.
[0016] (4) Litmus milk : coagulation was not accepted although the culture-medium color acidified. 72 hours after cultivating, liquefaction starts from a surface, and it is one third of culture media after one week. It became transparence.
[0017]
C. Physiological property (1) Gram's stain nature : + (2) Reduction ability of a nitrate : + (3) Aerosis [in denitrification reaction [nitrate bouillon]]: + (4) MR test : + (5) VP test : - Generation of (6) Indore : - (7) Generation of a hydrogen sulfide : - (8) Hydrolysis of starch : + (9) Use of a citric acid [a Simmns culture medium and a Christensen culture medium]: Use of the source of -(10) inorganic nitrogen [a nitrate and ammonium salt]: Generation of + (11) coloring matter: - (nonaqueous solubility) (12) Urease : - (13) oxidases : + (14) catalases : + range of (15) growth: Temperature; 45-70degree-C/ pH; Attitude against 5.0-7.0 (16) oxygen: aerotropism - a denominator -- anaerobic (17) O-F trial [Hugh leifson -- law]: Fructose (+) (18) Use and the acid of a saccharide, and gas generation : [0018]
[Table 2]

糖	酸			ガス	
	24時間	48時間	7日	48時間	7日
L-アラビノース	-	-	-	-	-
D-キシロース	-	-	-	-	-
D-グルコース	+			-	-
D-マンノース	-	+		-	-
D-フラクトース	-	-	-	-	-
D-ガラクトース	-	-	-	-	-
マルトース	-	+		-	-
シュークロース	-	+		-	-
ラクトース	-	-	-	-	-
トレハロース	-	+		-	-
D-ソルビトール	-	-	-	-	-
D-マンニトール	-	+		-	-
イノシトール	-	-	-	-	-
グリセリン	-	+		-	-
デンプン	-	-	-	-	-

[0019] Many properties of the above-mentioned new microorganism are Bacillus stearothermophilus. (Bacillus stearothermophilus) Since it corresponded well with many properties which it has, it is Bacillus stearothermophilus. It was named SPT 2-1 (Bacillus stearothermophilus SPT 2-1). In addition, Bacillus stearothermophilus obtained in the invention in this application An applicant for this patent ****s to 1-1-3, **, Higashi, Tsukuba-shi, Ibaraki-ken in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of the whereabouts, the Ministry of International Trade and Industry, on January 18, Heisei 8, and SPT 2-1 (Bacillus stearothermophilus SPT 2-1) is a trust number. FERM P-15395 It is given and even that variant and descendant that have the capacity mentioned above as well as this deposition microorganism itself mean the invention in this application.
[0020] Other modes and advantages of this invention are clear to below from the indication of the example shown in instantiation.

[Translation done.]

EXAMPLE

[Example]
Example 1: Bacillus stearothermophilus SPT 2-1 From the digested sludge in the aerobic thermophilic-digestion tub (70 degrees C) continuously operated for isolation three months, sludge was extracted to strain separation.
[0022] After diluting the extracted sludge with sterilized water suitably 1x10-3 to 10 to 5 times, it was applied to YP agar plate (product made from DIFCO). This plate was cultivated at 65 degrees C overnight, and the single colony was obtained.
[0023] the stock which checked the resolution of starch, the resolution of skim milk, and the resolution of sterilization sludge by the existence of the halo formation by the substrate mixing culture medium, and formed the halo about the obtained single colony -- especially -- sludge -- what has large resolution was selected as a description stock. In addition, assay of the resolution of starch and skim milk was based on the strange method of R.BEAUDET, C.GAGNON, J.G.BISAILLON and M.ISHAQUE, "Microbiological Aspects of Aerobic Thermophilic Treatment of Swine Waste", Applied and Environmental Microbiology, pp.971-976, and April 1990.
[0024] About these descriptions stock, the decomposition activity of sterilization sludge was examined further, and the stock with the highest activity was named two to one share of SPT. Moreover, the stock (SPT 1-6) in which the decomposition activity of sterilization sludge was shown similarly was selected as a stock for a comparison.
[0025] By 0.1N hydrochloric acid (Wako Pure Chem make) of Example 2:effects which pH and temperature over the activity of two to one share of SPT have, and/or 0.1-N sodium hydroxide (Wako Pure Chem make) YP culture medium (: made from DIFCO -- yeast extractives 4g --) adjusted to pH (pH 4.8, 5.0, 5.2, 5.5, 5.7, 6.8, 7.0) of the measuring object Peptone 8g and water 1 l;pH6.8 were prepared, strain (two to one share of SPT) was inoculated into this culture medium, and it cultivated at 70 degrees C for 6 hours. The growth cell mass of two to one share of SPT was observed, and whenever [that growth] was measured qualitatively. The result was shown in the following table 3.
[0026]
[Table 3]

pH	生育度
4.8	-
5.0	++
5.2	++
5.5	++
5.7	++
6.8	++
7.0	+

凡例： 生育度 (-) ：生育せず
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[0027] From the result of Table 3, it became clear that two to one share of SPT grew actively in pH of the range of pH 5.0- 6.8.

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[0029]
[Table 4]

温度	生育度
10	-
25	-
45	±
55	+
60	++
65	++
70	++
80	-

凡例： 生育度 (-) ：生育せず
(±) ：わずかに生育
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[0030] From the result of Table 4, it became clear that two to one share of SPT showed especially 45-70 degrees C of active growth conditions in a 60-70-degree C temperature requirement.
[0031] Example 3: Cultivate the assay sample offering strain (one platinum loop: two to one share of SPT, and one to six shares of SPT) of sludge solubilization effectiveness at 70 degrees C for 15 hours by the culture medium of YP culture medium (the

product made from DIFCO: yeast extractives 4g, peptone 8g, water 1 l;pH6.8). The cultivated strain was inoculated into sterilization sludge (initiation VM(Volatile Matter: all organic substances):11000ppm, VSS:11000ppm) so that it might become 1% (vol./vol.) of concentration, and shaking culture was performed at 70 degrees C. Shaking culture was finished, after fixed time amount (24 hours and 48 hours), sampling was performed, organic nature solid content in a sample (VSS:Volatile Suspended Solids) was measured, and the rate of solubilization was calculated from the decrement of solid content based on the following relational expression.

[0032]

[Equation 1]

$$\text{VSS可溶化率（%）} = \frac{\text{初発VSS} - \text{培養後VSS}}{\text{初発VSS}} \times 100$$

[0033] The result was shown in the following table 5 and drawing 1 . In addition, sterilization sludge was prepared according to the publication of "the development report (volume on sewerage) of the new waste-water-treatment system which utilized biotechnology", 73rd page - the 77th page, and Public Works Research Center (February, Heisei 3). Moreover, measurement of VSS was performed according to the test of sewage (1984 editions).

[0034]

[Table 5]

時間（日）		VSS可溶化率（%） 対照	SPT2-1	SPT1-6
0日	1回目	1	2.5	1
	2回目	−1	−2.5	−1
	平均	0	0	0
1日	1回目	10.4	18.8	15.2
	2回目	4.7	21.9	13.8
	平均	8	20.3	14.5
2日	1回目	7.4	38.1	10.4
	2回目	7.4	33.9	14.2
	平均	7.4	36	12.7

[0035] Example 4: Cultivate the assay sample offering strain of the solubilization effectiveness of **** resolvability starch content wastewater (starch industrial liquid waste) at 70 degrees C for 15 hours by the culture medium of YP culture medium (the product made from DIFCO: yeast extractives 4g, peptone 8g, water 1 l;pH6.8). The cultivated strain was inoculated into **** resolvability starch content wastewater (starch industrial liquid waste, initiation VM:10,000ppm, VSS:7,000ppm) so that it might become 1% (vol./vol.) of concentration, and shaking culture was performed at 70 degrees C for 24 hours, 48 hours, and 72 hours. Sampling was performed after each culture time amount, organic nature solid content (VSS) in a sample was measured (the test of sewage (1984 editions) shown above was followed), and the rate of solubilization was calculated from the decrement of solid content based on the following relational expression.

[0036]

[Equation 2]

$$\text{VSS可溶化率（%）} = \frac{\text{初発VSS} - \text{培養後VSS}}{\text{初発VSS}} \times 100$$

[0037] The result was shown in the following table 6 and drawing 2 .

[0038]

[Table 6]

時間（日）	VSS可溶化率（%） 対照	SPT2-1	SPT1-6
0日	0	0	0
1日	0.1	33.6	14.7
2日	-0.8	36.2	24.7
3日	0.2	50.4	36.8

[Translation done.]

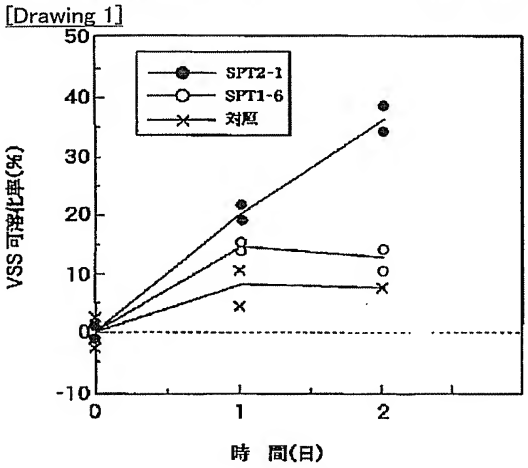
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Bacillus stearothermophilus SPT 2-1 It is the graph which shows the assay result of the solubilization effectiveness of sterilization sludge.
[Drawing 2] Bacillus stearothermophilus SPT 2-1 It is the graph which shows the assay result of the solubilization effectiveness of **** resolvability starch content wastewater solid content.

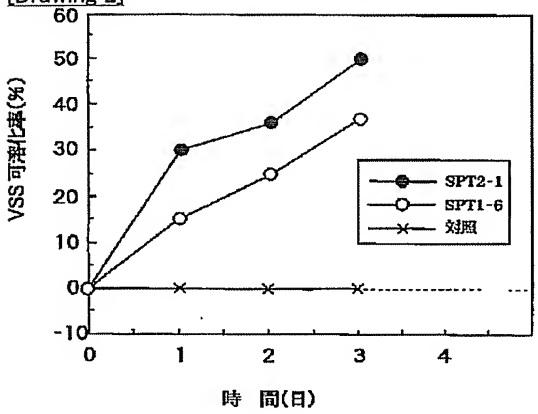
[Translation done.]

DRAWINGS

[Drawing 1]



[Drawing 2]



[Translation done.]